Environmental Health Perspectives
Vol. 99, pp. 253-255, 1993

Mainstream and Sidestream Cigarette Smoke-Induced DNA Adducts in C7Bl and DBA Mice

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Exposure to environmental tobacco smoke (ETS), which is largely composed of the sidestream cigarette smoke, has been implicated in increased incidence of cancer among nonsmokers. The present study was conducted to compare the potential of mainstream and sidestream cigarette smoke to induce DNA adducts in mice. Groups of female C57Bl and DBA mice were exposed twice daily for 65–70 weeks to mainstream or sidestream smoke from the University of Kentucky reference cigarettes (2R1) in a nose-only exposure system. Animals received a total particulate matter dose of about 16 and 6 mg/kg body weight/exposure and exhibited blood carboxyhemoglobin levels of about 16 and 34%, for mainstream and sidestream smoke-exposed groups, respectively. Pulmonary aryl hydrocarbon hydroxylase (AHH) activity was induced by about 2- to 3-fold in both mainstream and sidestream groups of C57Bl and in mainstream smoke-exposed group of DBA mice, but not in sidestream smoke-exposed DBA mice. An analysis of total DNA adduct levels by the ^{32}P-postlabeling assay showed a significant (12- to 25-fold) increase in the magnitude of preexisting lung DNA adducts in both mainstream and sidestream smoke-exposed C57Bl and DBA mice. Smoke exposures did not affect the total preexisting DNA adducts in liver of either strain. It is concluded that both mainstream and sidestream smoke are capable of enhancing preexisting DNA adducts in the lungs of chronically smoke-exposed mice.

Introduction

An association between cigarette smoking and human cancer has been well documented. A number of studies have reported the presence of DNA adducts in the tissues of human smokers and smoke-exposed animals (*1–5*). In recent years, epidemiological evidence has also implicated passive exposure to environmental tobacco smoke (ETS) with increased incidence of cancer among nonsmokers (*6*). The present study was conducted to compare the ability of mainstream and sidestream cigarette smoke to induce DNA adducts in the lung tissue of mice after chronic exposure. Two strains of mice known to differ in inducibility of their hepatic mixed-function oxidase system (*7*) were used to determine the formation of cigarette smoke-induced DNA adducts.

Experimental Procedures

Female C57Bl and DBA mice were purchased at age 8–9 weeks and housed in hanging, stainless-steel wire cages in environmentally controlled Bioclean rooms maintained under a daily light cycle of 12 hr and undergoing 40 air changes/hr to minimize the exposure of animals to extraneous particulates. Animals had free access to Purina rodent chow and water. After a 2-week quarantine period, the animals were randomly divided into four groups; *a*) room control (RC), handled once a week for cage cleaning; *b*) sham-treated (SH), given daily treatments identical to smoke-exposed groups but in the absence of smoke to simulate stress conditions; *c*) sidestream smoke-exposed (SS); and *d*) mainstream smoke-exposed (MS). Both smoke-exposed groups received daily exposures to either sidestream or mainstream smoke from the University of Kentucky reference cigarettes (2RI) in a nose-only exposure system for 65–70 consecutive weeks as described elsewhere (*8,10*).

Exposure of animals to smoke was monitored by measuring total particulate matter (TPM) intake, blood carboxyhemoglobin (COHb) levels, and the induction of pulmonary aryl hydrocarbon hydroxylase (AHH) as described previously (*8*).

DNA was isolated from individual lung and liver tissues of three animals in each group and analyzed by the nuclease P1 version of ^{32}P-postlabeling assay as described previously (*4,9*). DNA yields averaged 1.5–3.0 mg/g tissue. The data are expressed as attomoles of adducts per microgram DNA.

Results and Discussion

Daily TPM intake and blood COHb values demonstrated that both strains inhaled similar amounts of particulate and gas phase constituents of cigarette smoke (Table 1). The TPM received by

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Table 1. Blood carboxyhemoglobin and total particulate matter intake values of C57B1 and DBA mice.

Mouse strain	Treatments	COHb, %	TPM, mg/kg body weight
DBA	MS	16.7 ± 0.41	15.05 ± 0.42
	SS	32.5 ± 0.62	5.21 ± 0.25
C57B1	MS	18.0 ± 1.48	16.7 ± 1.62
	SS	35.37 ± 2.17	7.05 ± 0.62

Abbreviations: COHb, carboxyhemoglobin; TPM, total particulate matter; MS, mainstream smoke exposed; SS, sidestream smoke exposed.

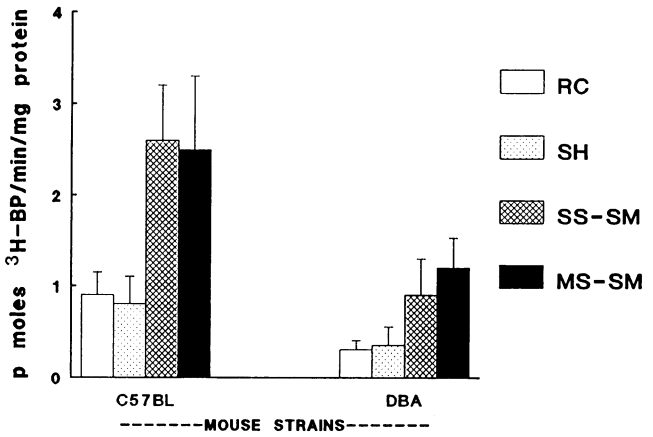

FIGURE 1. Induction of pulmonary aryl hydrocarbon hydroxylase (AHH) activity by mainstream (MS) and sidestream (SS) cigarette smoke in C57Bl and DBA mice.

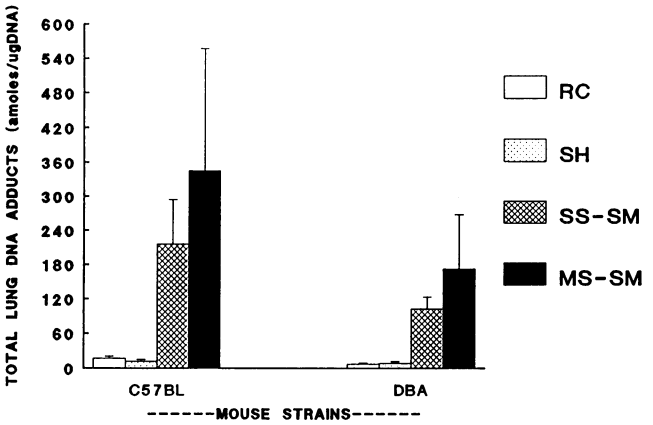

FIGURE 2. Total DNA adducts in the lung tissue of C57Bl and DBA mice after exposure to mainstream (MS) and sidestream (SS) cigarette smoke.

the SS group of both strains was approximately half that received by the MS group, but the COHb values in the SS groups were approximately twice that of the MS groups (Table 1). These data clearly indicated that both strains had inhaled smoke during exposures. The differences between SS and MS groups are consistent with our earlier observations (*10*) and reflect continuous and intermittent exposure of animals to sidestream and mainstream smoke, respectively.

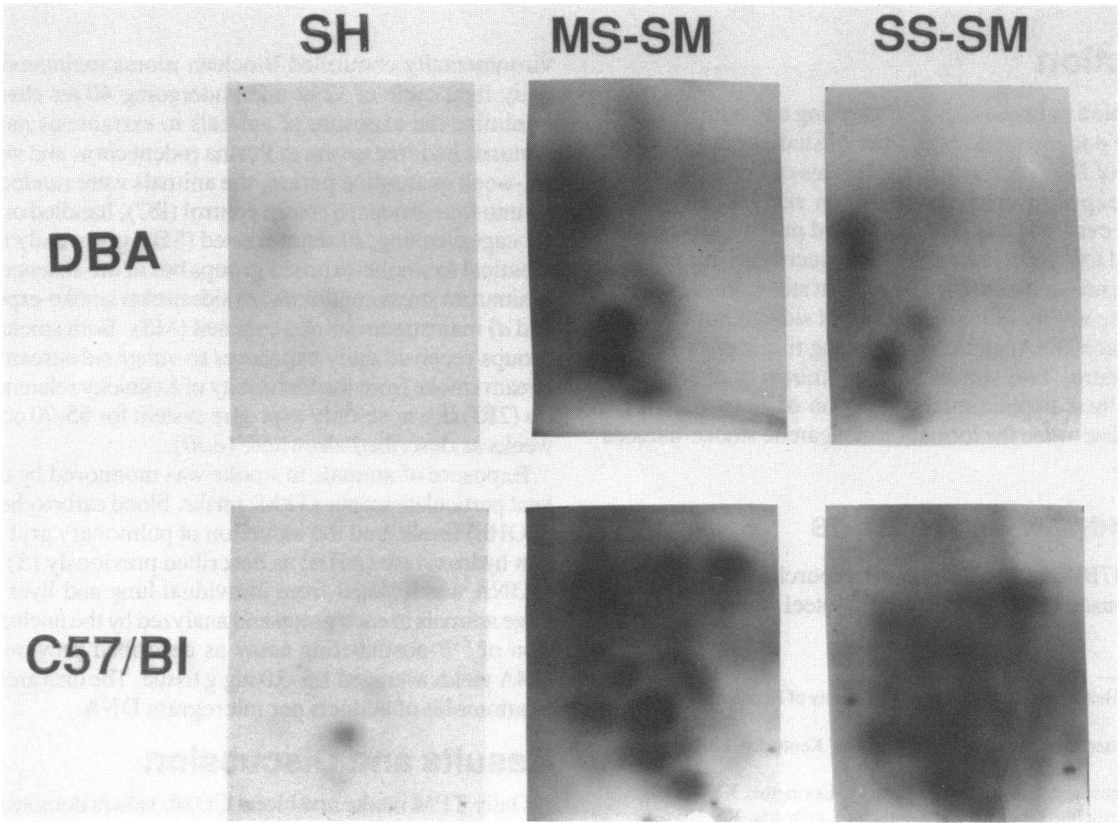

FIGURE 3. DNA adduct maps of lung tissue from C57Bl and DBA mice given chronic exposure to mainstream (MS) and sidestream (SS) cigarette smoke.

Exposure to sidestream and mainstream smoke induced pulmonary AHH activity by about 2- to 3-fold in C57Bl mice over controls. In DBA mice, a similar increase in pulmonary AHH was seen in MS group but such increase in SS group was not statistically significant (Fig. 1).

DNA adduct analyses showed that both types of cigarette smoke enhanced preexisting DNA adducts in mice lungs. The total DNA adduct levels in MS and SS groups of both mouse strains were several-fold higher than their respective room and sham-treated controls (Fig. 2). While the adduct levels in SS groups of both strains were generally lower than the MS groups, the differences were not statistically significant. The total adduct levels in smoke-exposed animals of DBA strain were also generally lower than C57BL mice, but again these differences were not statistically significant, possibly due to large interanimal variation within each group. Adduct maps also did not show qualitative differences between strains or between sidestream and mainstream groups (Fig. 3). Furthermore, by using larger DNA samples and extending the exposure duration of radiochromatograms to film, it could be demonstrated that the adduct spots present in smoke-exposed mice lungs were also present in the control animals. These observations suggested that cigarette smoke exposures simply increased the magnitude of already existing adducts without inducing newer DNA lesions. An enhancement of preexisting DNA adducts by cigarette smoke and an absence of qualitative differences in adduct maps of mice lung DNA are consistent with our earlier data in rats (*4,9*).

The observations described above suggest that both sidestream and mainstream cigarette smoke are capable of enhancing preexisting DNA adducts in mice lungs. Therefore, the presence of enhanced DNA adducts in tissues may be used as a biomarker for either type of cigarette smoke exposure.

This manuscript was presented as a poster at the Conference on Biomonitoring and Susceptibility Markers in Human Cancer: Applications in Molecular Epidemiology and Risk Assessment that was held in Kailua-Kona, Hawaii, 26 October–1 November 1991.

This work was supported by a U.S. Environmental Protection Agency cooperative agreement (CR-816/85 to R.C.G.) and a KTRB grant (5-41110 to C.G.G.).

REFERENCES

1. Weinstein, I. B. Cigarette smoking and its fingerprint on DNA. J. Natl. Cancer Inst. 80: 548–549 (1988).
2. Phillips, D. H., Hewer, A., Martin, C. N., Garner, R. C., and King, M. M. Correlation of DNA adduct levels in human lung with cigarette smoking. Nature 336: 790–792 (1988).
3. Talaska, G., Al-Juburi, A. Z. S. S., and Kadlubar, F. F., Smoking related carcinogen-DNA adducts in biopsy samples of human urinary bladder: identification of N-(deoxyguanosin-8-yl)-4-aminobiphenyl as a major adduct. Proc. Natl. Acad. Sci. U.S.A. 88: 5350–5354 (1991).
4. Gupta, R. C., Sopori, M. L., and Gairola, C. G. Formation of cigarette smoke-induced DNA adducts in the rat lung and nasal mucosa. Cancer Res. 49: 1916–1920 (1989).
5. Bond, J. A., Chen, B. T., Griffith, W. C., and Mauderly, J. L.; Inhaled cigarette smoke induces the formation of DNA adducts in lungs of rats. Toxicol. Appl. Pharmacol. 99: 161–172 (1989).
6. U.S. Department of Health and Human Services. The Health Consequences of Involuntary Smoking: A Report of the Surgeon General CDC 87-8398, U.S. Government Printing Office, Washington, DC, 1986, pp 182–246.
7. Abramson, R. K. and Hutton, J. J. Effects of cigarette smoking on aryl hydrocarbon hydroxylase activity in lungs and tissues of inbred mice. Cancer Res. 35: 23–29 (1975).
8. Gairola, C. G. Free lung cell response of mice and rats to mainstream cigarette smoke exposure. Toxicol. Appl. Pharmacol. 84: 567–575 (1986).
9. Gairola, C. G. and Gupta, R. C.; Cigarette smoke-induced adducts in the respiratory and nonrespiratory tissues of rats. Environ. Mol. Mutagen. 17: 253–257 (1991).
10. Gairola, C. Pulmonary aryl hydrocarbon hydroxylase activity of mice, rats and guinea pigs following longterm exposure to mainstream and sidestream cigarette smoke. Toxicology 45: 177–184 (1987).